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Original article

Exhaled nitric oxide predicts airway hyper-responsiveness to hypertonic saline in children that wheeze

Background: Exhaled nitric oxide (eNO) has shown good validity for the assessment of airway inflammation in asthmatic children. In large-scale epidemiological studies, this method would be preferred above airway challenge tests, because it is a quick and easy applicable tool.

Objective: In this study, we aimed to assess the discriminatory capacity of eNO, and prechallenge FEV₁ for airway hyper-responsiveness (AHR) in 8–13-year old schoolchildren.

Materials and methods: Parents completed the ISAAC questionnaire, and children were tested for atopy, AHR to hypertonic (4.5%) saline (HS), and eNO. Diagnostic value was assessed by the area under the receiver operating curves (ROC), and calculation of positive and negative predicted values at different cut-off points for eNO and prechallenge FEV₁.

Results: Areas under the ROC-curves of AHR were 0.65 for eNO and 0.62 for FEV₁. Values increased to 0.71 and respectively 0.75 for a combined occurrence of AHR and current wheeze. Highest sensitivity and specificity were obtained at a cut-off value of 43 ppb for eNO and 103% predicted for FEV₁. At these cut-off values, the positive predictive values for the presence of AHR in symptomatic children were respectively 83% (eNO) and 33% (FEV₁), and negative predictive values in asymptomatic children were, respectively, 90 (eNO) and 80% (FEV₁).

Conclusion: Exhaled nitric oxide is a valid screening tool for AHR to HS in children that present with current wheeze, and it outperforms FEV₁ as a predictor of AHR.

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Key words: airway hyper-responsiveness; exhaled nitric oxide; predictive values; screening.

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Airway hyper-responsiveness (AHR) is considered an objective measure of airway liability, which is a hallmark of asthma. The assessment of AHR has become common practice in epidemiological studies, since steering committees of the European Community Respiratory Health Survey (ECRHS) and the International Study on Allergy and Asthma in Childhood (ISAAC) have adopted standards for airway challenge tests with methacholine and hypertonic saline (HS) (1, 2). The worldwide use of these protocols has given new insights in the features of asthma and allergic disease, and environmental factors that play a role. Future studies should include the evaluation of community-based intervention strategies on the development and treatment of asthma. To this end, screening tools are needed to select subjects with and without asthma features. However, airway challenge testing which is considered the 'gold standard' is time consuming and needs full cooperation of the subject. In fact, the test is inefficient and expensive when used in a community-based design with most subjects not being hyper-responsive.

There is a need of alternative tools to monitor processes involved in AHR. The efficiency of community-based studies including AHR may otherwise be increased by

preselection of subjects at increased risk of being hyper-responsive. Assessment of nitric oxide in exhaled breath (eNO) seems attractive, since it is quick and easy to perform, both for participant and researcher. In asthma patients eNO level is associated with lung function, symptom score, and bronchial inflammation (3–8). Furthermore, eNO decreases in response to anti-inflammatory treatment consistent with a decline in symptoms, sputum eosinophil numbers, and PC₂₀ methacholine (9–12). Although most studies have focused on eNO as an inflammatory marker, it is related to airway remodeling as well (13).

Atopy seems to play a crucial role in the asthma phenotype that is reflected by elevated eNO. Patients with atopic asthma show higher eNO levels, whereas similar values are observed in nonatopic asthma patients and healthy controls (14). Moreover, in community-based studies an association of eNO with AHR has been demonstrated only in atopic subjects (15, 16).

The close relationship between atopy and asthma in children suggests that eNO may be used as a screening tool for AHR. To this aim, we have evaluated the discriminatory characteristics of eNO for the presence of

AHR to HS in a community-based sample of 8–13-year old schoolchildren. A comparison was made for the discriminatory capacity of eNO and FEV₁ % predicted.

Materials and methods

Study population and design

The population comprised of 2207 school children aged 8–13 years that participated in a cross-sectional survey on respiratory health effects of living close to a freeway. A detailed description of the protocol has been published previously (17). Shortly, the protocol comprised of the ISAAC questionnaire on respiratory health ($N = 2207$), assessment of atopic status by skin prick testing (SPT) or serum IgE ($N = 1413$), and airway challenge testing ($N = 1404$). Exhaled nitric oxide was assessed in a random sample ($N = 436$). The parents gave Informed consent for each test separately. The Medical Ethical Board of the University of Wageningen approved the study protocol.

Questionnaire

By ISAAC core questionnaire, data were collected on 'current wheeze' (i.e. wheeze in the past 12 months) and 'ever asthma.'

Atopy

Skin prick testing (ALK, the Netherlands) and measurement of serum IgE (CAP-assay, Pharmacia, the Netherlands) occurred for mixed grass and tree pollen, cat, dog, house dust mites (*Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*), and mixed moulds. Atopy was defined by a mean wheal diameter ≥ 3 mm at SPT, or serum specific IgE ≥ 0.35 kU/l.

Lung function and bronchial challenge

Airway challenge to HS (4.5%) was performed according to the ISAAC-phase 2 standards. Salbutamol was withheld for 6 h, antihistaminic and cromoglycate for 48 h. None of the children used long-acting beta-agonists. Lung function was assessed according to ERS guidelines using a pneumotachometer (Jaeger, Germany) (18). The protocol comprised of inhalation HS aerosol for subsequently 0.5, 1, 2, 4 and 8 min, and two reproducible measurements of FEV₁ were achieved after each inhalation step. The test stopped if FEV₁ fell 15% or more, or after completing all inhalation steps. The amount HS inhaled was assessed by the weight difference of the saline canister. PD₁₅HS was determined by linear interpolation between the last two data points of the dose-response curve, and AHR was defined by a PD₁₅HS ≤ 23.0 ng.

Of the 1404 participants, 1159 children completed the test satisfactory. Excluded from bronchial challenge testing were children with a prechallenge FEV₁ < 75% predicted ($N = 17$) (19) and those that were unable to perform technically satisfied forced maneuvers ($N = 130$). Another 98 tests were discarded because of excessive cough, unwillingness, and inability to perform acceptable spirometry during the challenge test.

Exhaled nitric oxide

Exhaled air was sampled in 1000 ml foil balloons (Mylar balloon, ABC ballonnen, Zeist, the Netherlands). Exhaled air was sampled

with a sampling device made up of a rigid tube connecting a mouthpiece and a sampling balloon, equipped with a calibrated narrowing in the exhalation circuit as described previously (20). The first 750 ml of exhaled air putatively contaminated with ambient NO was collected at high-exhalation flow rate in the first bag, connected via a wide-bore T-piece of the rigid tube of the sampling device, and discarded. The second bag was filled over a total time of 20 s with air exhaled 500 ml/min and exhalation backpressure of 20 cm water pressure. Analysis occurred within 3 h after sampling, as we have shown previously that eNO concentrations in the sampling balloons were stable for 18 h.

Exhaled air was collected at a flow rate of 500 ml/min (8.3 ml/s) at 20 cm H₂O backpressure. At younger age, children may have difficulties to exhale during 20 s. Indeed, some of our children (<2%) had to repeat the maneuvers for a second or third time. As such, we had no dropouts. We preferred a low-flow rate aiming to increase sensitivity and allowing assessment of low-eNO concentrations, as expected to be common in a community-based population of mostly healthy subjects (21, 22). Although our method differs from ERS and ATS recommendations (23, 24), it correlates well with recommended methods (21). Duplicate analyses of eNO occurred by a rapid response chemiluminescence analyzer (Sievers 280B, Boulder) with a response time of <200 ms, and a sample flow rate of 200 ml/min. Duplicate eNO measurement showed good repeatability [standard deviation (SD) <5%].

Statistical analysis

For the current analysis, the population was restricted to children with complete data on symptoms, atopic sensitization, airway challenge testing, and eNO. Predictors were selected using step-wise logistic regression with backward elimination of co-variables with $P > 0.157$, according to Akaike's Information Criterion for selection with all co-variables with one degree of freedom (25). The final reduced models included variables with $P < 0.05$.

Test performance was estimated by the area under ROC (AUC). Generally, discrimination is considered acceptable if AUC > 0.7 (26). Dichotomization of eNO and FEV₁ occurred by values with the highest sum of sensitivity and specificity. At this value, positive and negative predictive values were assessed.

Results

Complete data on questionnaire, atopic sensitization, airway challenge, and eNO were obtained for 201 children. Characteristics of children included in the current analyses were equal to children eligible to have been included, i.e. aged 8 and older (Table 1).

Both eNO and FEV₁ % predicted were associated with AHR in bivariate logistic regression analysis (Table 2). The multivariate regression model included maternal asthma ($P = 0.14$), a child's history of ever asthma, current wheeze, and the presence atopy (all $P < 0.001$). Determinants with $P < 0.05$ were included in the reduced model, i.e. current wheeze and atopy. Adding eNO or FEV₁ % predicted resulted in different prediction models with atopy and current wheeze included for FEV₁, and only current wheeze for eNO.

Receiver operating curves analyses are presented in Fig. 1 and Table 3. The prediction AHR by a single

Table 1. Characteristics of children included and not included in the current analysis

	Included (N = 201)		Not included	
	n	%	n/N	%
Girls	94	53.2	1022/2006	51.0
Mother asthma	16	8.2	143/1966	7.3
Father asthma	12	6.6	122/1822	6.7
Atopy	62	30.8	391/1212	32.3
Current wheeze	35	17.4	348/1958	17.8
Ever asthma	17	8.6	162/1975	8.2
AHR	37	18.4	199/958	20.8

AHR, airway hyper-responsiveness.

assessment of eNO (Fig. 1A) or FEV₁ % predicted (Fig. 1B) yielded an AUC of 0.64 and respectively 0.63 (Table 3). Adding current wheeze increased the AUC for eNO and FEV₁ to 0.71 and respectively 0.75. Including atopy as well did not change the results essentially, and neither did inclusion of both eNO and FEV₁ % predicted.

Highest sensitivity and specificity were obtained at cut-off values of 39 and 43 ppb for eNO, and 103% predicted for FEV₁ (Table 4). The cut-off value for eNO was arbitrary set at 43 ppb.

Table 5 presents positive and negative predictive values for AHR of eNO and FEV₁ % predicted at cut-off levels of 43 ppb and 103%. A positive test result for current wheeze, eNO (> 43 ppb), or FEV₁ (≤ 103% predicted) predicted the presence of AHR in less than 50%. Preselection by the presence of current wheeze increased the positive predictive values to 83% for eNO and 33% for FEV₁. The absence of AHR was correctly predicted in 87, 88 and 74% for the absence of current wheeze, eNO ≤ 43 ppb, and FEV₁ > 103% predicted, respectively. Results did not change essentially after preselection of children without current wheeze. Predictive values increased at higher cut-off levels for eNO or lower cut-off levels for FEV₁.

Figure 1. Receiver operating characteristic curves from multivariate logistic regression analyses for the prediction of airway hyper-responsiveness (AHR) to hypertonic saline. (A) eNO with or without current wheeze (whe) and atopy (ato). (B) FEV₁ % predicted with or without current wheeze (whe) and atopy (ato).

Table 2. Associations with airway hyper-responsiveness (AHR) by logistic regression analysis, OR (95% CI)

	Bivariate model	Reduced multivariate model	Reduced model + eNO	Reduced model + FEV ₁ % predicted
ENO (ppb)	1.02 (1.01, 1.04)‡	Not included	1.01 (1.00, 1.03)*	Not included
FEV ₁ , % predicted	0.96 (0.92, 1.00)*	Not included	Not included	0.95 (0.92, 0.99)*
Age (years)	1.16 (0.86, 1.56)	Not included	Not included	Not included
Male	1.19 (0.58, 2.44)	Not included	Not included	Not included
Father asthma	1.63 (0.42, 6.40)	Not included	Not included	Not included
Mother asthma	2.34 (0.76, 7.23)	Not included	Not included	Not included
Ever asthma	6.15 (2.19, 17.29)‡	Not included	Not included	Not included
Current wheeze	5.82 (2.59, 13.04)‡	4.43 (1.90, 10.29)‡	4.05 (1.69, 9.72)†	4.95 (2.06, 11.89)‡
Atopy	3.94 (1.88, 8.26)‡	2.95 (1.35, 6.45)†	2.10 (0.89, 4.98)	2.83 (1.27, 6.30)*

* $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$.

eNO, exhaled nitric oxide.

Table 3. Area under the receiver operating curves (AUC) for detection of airway hyper-responsiveness (AHR) by exhaled nitric oxide (eNO) and FEV₁ % predicted

	eNO	FEV ₁	eNO + FEV ₁
Single test	0.65	0.62	0.71
+ Current wheeze	0.71	0.75	0.77
+ Current wheeze + atopy	0.73	0.77	0.77

Table 4. Sensitivity and specificity for airway hyper-responsiveness (AHR) at different cut-off levels of exhaled nitric oxide (eNO) and FEV₁

Exhaled nitric oxide			FEV ₁ % predicted		
Cut-off	Sensitivity	Specificity	Cut-off	Sensitivity	Specificity
31	30	93	91	16	89
33	32	92	93	27	83
35	32	90	95	35	76
37	38	90	97	43	68
39	43	90	99	43	65
41	43	87	101	60	60
43	49	84	103	76	52
45	51	80	105	76	46
47	54	76	107	81	37
49	nd	nd	109	84	30
51	nd	nd	111	86	23
53	54	66	113	92	16
55	57	61	115	95	11

nd, not determined.

Table 5. Percent predictive values of exhaled nitric oxide (eNO) and FEV₁ for the presence of airway hyper-responsiveness (AHR)

	Positive predictive value (%)	
	All children	Children with current wheeze
Current wheeze = 'yes'	46	—
eNO >43 ppb	41	83
FEV ₁ ≤ 103 % predicted	10	33
	Negative predictive value (%)	
	All children	Children with no current wheeze
Current wheeze = 'no'	87	—
eNO ≤ 43 ppb	88	90
FEV ₁ >103 % predicted	74	80

Discussion

Our study shows that a single measurement of eNO has a high predictive value for the presence of AHR to HS in school children with a history of wheeze in the past 12 months, whereas it excludes AHR in the majority of children without wheeze. However, the positive predictive value is low if not taking into account a history of wheeze. Prechallenge FEV₁ insufficiently discriminates for AHR,

irrespective whether a history of current wheeze is taken into account.

Previous studies have shown that eNO is associated with eosinophilic inflammation (27), and predicts steroid responsiveness in bronchial asthma (28). In this study, we aimed to evaluate eNO as a screening tool for AHR in a community-based population. Airway challenge tests are considered the 'gold standard,' but are time consuming and need full cooperation of the subject. Moreover, efficiency is low since most subjects are not hyper-responsive. We suppose that epidemiological studies may benefit from a screening tool that discriminates between subjects with and without AHR.

Like others, we found higher eNO and lower FEV₁ in children with AHR (29, 30). The predictive model additionally included current wheeze and atopy that were independently associated with AHR as well. According to ROC-analyses, discrimination of AHR was insufficient by eNO or prechallenge FEV₁ alone. Including current wheeze yielded acceptable discriminatory capacity (>0.7) both for eNO and prechallenge FEV₁. This confirms previous results that eNO discriminates asthma in subjects referred to a clinic because of symptoms (31). We observed a similar discrimination for the combination of atopy and eNO or FEV₁, though preferred questionnaire-based data because of the more easy and feasible availability compared to atopic sensitization.

The higher discrimination by eNO for a joint presence of AHR with wheeze may be due to a joint presence of atopy, as previous studies have shown a relationship with AHR in atopic but not in nonatopic children (15, 16). Conversely, the associations of eNO with both wheeze and atopic sensitization may reflect allergic inflammation as common pathway. In this population, we have previously shown an association between AHR and blood eosinophilic markers only if recent wheeze was present as well (32). Taken together, the observations of a higher eNO in atopics, the common presence of atopy in childhood asthma and eosinophilic inflammation if AHR is accompanied by recent symptoms, it is not surprising that eNO predicts AHR in symptomatic children.

Although the overall discriminatory power was similar for the combination of current wheeze and eNO or prechallenge FEV₁, figures differed considerably after dichotomization at the optimum cut-off point. In children with current wheeze, a high eNO predicts 83% of AHR whereas this is only 33% for a low prechallenge FEV₁. This is explained by the difference in shape of the ROC-curves that show a greater increase in sensitivity per unit specificity for eNO compared to prechallenge FEV₁. Our observations clearly show ROC-analysis alone is insufficient to assess discriminatory power.

The applicability of eNO as a screening tool for AHR needs further assessment in larger community-based populations, since this is a relatively small sample with 37 children having AHR and 35 having wheeze. Bias may have been introduced due to inaccurate report of wheeze

without distinguishing between asthmatic and nonasthmatic wheeze. This may have resulted in an excess report of nonasthmatic, or non-atopic, wheeze with consequently underestimation of the relationship between eNO and AHR.

Selection bias may have been introduced due to our selection criterion for airway challenge testing of a prechallenge FEV₁ >75% predicted. However, we do not think this will have played a relevant role. As none of the children with NO assessment had a prechallenge FEV₁ < 75% predicted.

As eNO is influenced by airway remodeling as well (13), the test performance may be influenced by the number of patients with long-standing asthma. In this study, however, no data were collected on the time of asthma diagnosis or duration of symptoms.

At a cut-off level of 43 ppb for eNO, 83% of children with current wheeze were correctly classified as having AHR, and 90% of the asymptomatic children as having no AHR. Although eNO is not similar to AHR, it may be considered a useful screening tool for AHR to HS in children presenting with wheeze in the past 12 months.

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